

Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

10/1009966

Applicant's or agent's file reference R30024PCT(M)	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/DE00/01944	International filing date (day/month/year) 13 June 2000 (13.06.00)	Priority date (day/month/year) 12 June 1999 (12.06.99)
International Patent Classification (IPC) or national classification and IPC C12N 15/82		
Applicant ROITSCH, Thomas		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 03 January 2001 (03.01.01)	Date of completion of this report 17 September 2001 (17.09.2001)
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/DE00/01944

I. Basis of the report

1. With regard to the elements of the international application:*

- ☐ the international application as originally filed
- ☒ the description:
 pages 1-34, as originally filed
 pages _____, filed with the demand
 pages _____, filed with the letter of _____
- ☒ the claims:
 pages _____, as originally filed
 pages _____, as amended (together with any statement under Article 19
 pages _____, filed with the demand
 pages 1-44, filed with the letter of 20 August 2001 (20.08.2001)
- ☒ the drawings:
 pages 1/22-22/22, as originally filed
 pages _____, filed with the demand
 pages _____, filed with the letter of _____
- ☒ the sequence listing part of the description:
 pages 1-11, as originally filed
 pages _____, filed with the demand
 pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

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IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos. _____

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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1 - 13, 15, 16, 18, 26 - 29, 31, 32, 34 - 37, 44	YES
	Claims	17, 19 - 25, 30, 33, 38 - 43	NO
Inventive step (IS)	Claims	1 - 13, 15, 16, 18, 26 - 29, 31, 32, 34 - 37	YES
	Claims	44	NO
Industrial applicability (IA)	Claims	1 - 13, 15 - 44	YES
	Claims		NO

2. Citations and explanations

See the Supplemental Box.

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Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: I, IV, V, VIII

1. This report makes reference to the following documents:

D1: WO-A-98/59061

D2: WO-A-98/41643

D3: GREINER ET AL: PLANT PHYSIOLOGY, Vol. 116, 1998,
pages 733 - 742

D4: ROITSCH THOMAS ET AL: PLANT PHYSIOLOGY, Vol. 108,
1995, pages 285 - 294

D5: MARIANI C ET AL: NATURE, Vol. 347,
25 October 1990, pages 737 - 741

Box I

Basis of the report

2. The amendments submitted with the letter of 20.08.01 introduce substantive matter which, contrary to PCT Article 34(2)(b), goes beyond the disclosure in the international application as filed. The amendments concerned are those in Claim 14. The original application did not disclose nucleic acid constructs which code for an invertase "that is derived from the organism belonging to the species into which the nucleic acid construct is to be introduced". (In addition, Claim 14 is unclear, because it does not mention any features of the claimed nucleic acid construct.)

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Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: I, IV, V, VIII

Box IV**Lack of unity of invention**

3. The International Preliminary Examining Authority agrees with the objection raised by the International Searching Authority concerning lack of unity of invention, because the claims relate to the following two different inventions listed in the international search report:

- (1) Claims 1 - 5 (wholly); 7 - 44 (in part)
Nucleic acid sequence of a tapetum- and pollen-specific promoter comprising SEQ ID NO: 1 or 2 (invertase derived from *Nicotiana tabacum*), expression system, nucleic acid constructs, vectors, cells, plants, seeds, fruit, hybrid seeds, method for producing male sterile plants, restorer plants, method for cloning promoters
- (2) Claims 6 (wholly); 7 - 44 (in part)
corresponding to invention 1, but comprising SEQ ID NO: 3 (invertase derived from tomatoes)

Document D1 discloses a tapetum- and pollen-specific promoter. The problem to be solved by the present application, having regard to that prior art, is to provide further such promoters. The claimed solutions (1) and (2) to this problem have no technical feature in common which, in the light of the prior art, can be regarded as a special technical feature within the meaning of PCT Rule 13.2.

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: I, IV, V, VIII

Consequently, the conclusion that must be drawn is that there is no single inventive concept that could unify the inventions contained in the application.

Box V

Reasoned statement under PCT Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

4. NOVELTY

The present application does not satisfy the criterion stipulated in PCT Article 33(2), because the subjects of Claims 17, 19 - 25, 30, 33 and 38 - 43 are not novel.

The subjects of Claims 17, 19 - 25, 30, 33 and 38 - 43 lack novelty over generally known cells and plants that contain the cited nucleic acids naturally. These claims do not define and clear distinguishing features. Basically, only clear features can establish novelty. In addition, a product is not rendered novel merely by the fact that it is produced by means of an (allegedly) new process.

5. INVENTIVE STEP

- 5.1. The subjects of Claims 1 - 13, 15, 16, 18, 26 - 29, 31, 32 and 34 - 37 are considered to involve an inventive step. Document D1 is regarded as the

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Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: I, IV, V, VIII

closest prior art. The technical problem was to provide further tapetum- and pollen-specific promoters. The solutions to this problem according to the above-mentioned claims were not obvious, having regard to the cited prior art.

- 5.2. The subject matter of the unclear Claim 44 (see below) is not considered to involve an inventive step, firstly because the term "functionally homologous" is unclear and secondly because it was known from D2 that invertase promoters are expressed in anthers.

In addition, it should be noted that the use of the oligonucleotides OlN3 and OlN4 for cloning invertases was already disclosed in D4.

Box VIII**Certain observations on the international application**

6. The expressions "more particularly", "preferred" and "preferably" in Claims 26, 27, 30 - 35, 39, 42 and 44 make these claims unclear, because they have no limiting effect and are therefore confusing in these claims.

In addition, the use of the vague and indefinite expressions "part of a nucleotide sequence" (Claims 9, 10, 12), "heterologous or homologous origin" (Claims 37 - 39) and "functionally homologous"

.../...

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(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: I, IV, V, VIII

(Claim 44) make the claims unclear (PCT Article 6). Claim 15 is unclear, because it does not define any features of the claimed nucleic acid construct. The feature whereby a plant comprises an "additional invertase" which "is different from the cell's own invertase" is unclear (Claims 30, 33), because plants generally contain several invertases.

Claim 18 appears to contain all the features of Claim 17 and is therefore not correctly worded as a dependent claim on the latter (PCT Rule 6.4).

7. Claim 25 defines a product in terms of the process for producing it. However, the claim is only considered to be clear if the application contains no other information that would enable the application to adequately characterize the product in terms of its composition, its structure or other verifiable parameters. In the present case, however, it is possible to define the products in terms of structural features.

Claims 24, 40, 42 and 43 ("seeds of a plant", "fruit of a plant") are likewise unclear, because the technical features of the claimed seeds or fruit are not defined.

CLAIMS

REPLACED BY
ART 34 AMDT

1. Nucleic acid sequence coding for a promoter, the promoter being both tapetum-specific and pollen-specific.
2. Nucleic acid sequence coding for a promoter, the nucleic acid sequence comprising a range of at least approximately 900 nucleotides upstream of the TATA box of the sequence represented in SEQ.ID. No. 1.
3. Nucleic acid sequence according to claim 2, characterized in that the nucleic acid sequence comprises a range of at least approximately 1000 nucleotides upstream of the TATA box of the sequence represented in SEQ.ID. No. 1.
4. Nucleic acid sequence according to claim 2 or 3, characterized in that the nucleic acid sequence comprises a range of at least approximately 1500 nucleotides upstream of the TATA box of the sequence represented in SEQ.ID. No. 1.
5. Nucleic acid sequence according to one of the claims 2 to 4, characterized in that the nucleic acid sequence comprises the sequence represented in SEQ.ID. No. 1.
6. Nucleic acid sequence coding for a promoter, the nucleic acid sequence comprising the sequence represented in SEQ.ID. No. 2.
7. Nucleic acid sequence coding for a promoter, the nucleic acid sequence comprising the sequence represented in SEQ.ID. No. 3.
8. Expression system comprising at least one nucleic acid according to one of the claims 1 to 7.
9. Expression system according to claim 8 comprising at least one terminator and/or a linker.
10. Nucleic acid construct comprising a nucleic acid sequence according to one of the claims 1 to 7 and at least part of an expressible nucleic acid sequence.
11. Nucleic acid construct according to claim 10, characterized in that the part of the expressible nucleic acid sequence or the complete, expressible sequence is linked in the sense direction with the nucleic acid sequence according to one of the claims 1 to 7.
12. Nucleic acid sequence according to claim 10 or 11, characterized in that

the expressible nucleic acid codes for an invertase.

13. Nucleic acid construct according to claim 12, characterized in that the part of the nucleic acid sequence of an invertase or the complete sequence of an invertase is linked in the antisense direction with the nucleic acid sequence according to one of the claims 1 to 7.

14. Nucleic acid construct according to one of the claims 12 and 13, characterized in that the invertase is of the type present in a structure selected from the group comprising anthers, tapetum, pollen precursor cells and pollen.

15. Nucleic acid construct according to one of the claims 10 to 14, characterized in that the invertase comes from the organism into which or into whose cells the nucleic acid construct is to be introduced and in particular comes from the plant group including the species into which the nucleic acid construct is to be introduced.

16. Nucleic acid construct according to one of the claims 10 to 15, characterized in that the organism is selected from the group comprising food plants, ornamental plants and medicinal plants.

17. Vector comprising a nucleic acid sequence according to one of the claims 1 to 7 and/or an expression system according to one of the claims 8 and 9 and/or a nucleic acid construct according to one of the claims 10 to 16.

18. Cell, particularly plant cell, comprising a nucleic acid according to one of the claims 1 to 7 and/or an expression system according to one of the claims 8 and 9 and/or an nucleic acid construct according to one of the claims 10 to 16 and/or a vector according to claim 17.

19. Cell, particularly according to claim 18, characterized in that the cell comprises a nucleic acid sequence according to one of the claims 1 to 7, which is a promoter, and a nucleic acid coding for an inhibitor of an invertase, the promoter controlling the expression of the inhibitor.

20. Cell according to claim 17 or 18, characterized in that the cell is selected from the group comprising pollen cells, pollen precursor cells and tapetum cells.

21. Cell according to one of the claims 18 to 20, characterized in that the cell is an arrested pollen cell.

22. Plant comprising a cell according to one of the claims 18 to 21.

23. Plant according to claim 22, characterized in that the plant is selected from the group comprising food plants, ornamental plants and medicinal plants and is preferably selected from the group comprising rice, maize, potatoes, tomatoes, rape, soya and sugar beet.

24. Plant according to claim 22 or 23, characterized in that the plant is a male, sterile plant and has at least one further modification of its genotype, particularly a change caused by genetic engineering.

25. Seed obtained from a plant according to one of the claims 22 to 24.

26. Hybrid seed obtainable in that a male, sterile plant according to one of the claims 22 to 24 is hybridized with another male, fertile plant and from such a filial generation the hybrid seed is obtained.

27. Process for the production of male, sterile plants, characterized in that a nucleic acid construct according to one of the claims 12 to 16 is introduced into a cell, particularly into a plant cell and based on this cell a plant is produced.

28. Process according to claim 27, characterized in that the plant is selected from the group comprising food plants, ornamental plants and medicinal plants and is preferably selected from the group comprising rice, maize, potatoes, tomatoes, rape, soya and sugar beet.

29. Use of a nucleic acid construct according to one of the claims 12 to 16 for producing sterile, male plants.

30. Use of a nucleic acid sequence according to one of the claims 1 to 7 for the expression of a nucleic sequence.

31. Restorer plant, characterized in that in a cell and preferably in a plurality of its cells, it comprises a nucleic acid according to one of the claims 1 to 7 as a promoter and a nucleic acid coding for a further invertase and which is controlled by said promoter, the further invertase differing from the cell's own invertase.

32. Restorer plant, preferably according to claim 31, characterized in that in a cell, preferably in most of its cells, it comprises a nucleic acid according to one of the claims 1 to 7 as a promoter and a nucleic acid coding for a saccharose transport system and which is controlled by this promoter.

33. Restorer plant according to claim 32, characterized in that in a cell and preferably in most of its cells, it comprises a nucleic acid according to one of the claims 1 to 7 as a promoter and a nucleic acid coding for saccharose

synthase and/or cytoplasmically expressed invertase and whose expression is controlled by the promoter.

34. Plant, characterized in that, in at least one cell and preferably in most of its cells, it comprises a nucleic acid construct according to one of the claims 12 to 16 and the cell or cells also comprise a nucleic acid sequence according to one of the claims 1 to 7 as promoter and a nucleic acid coding for a further invertase and which is controlled by the promoter, the further invertase differing from the cell's own invertase.

35. Plant, preferably according to claim 34, characterized in that in at least one cell and preferably in most of its cells, it comprises a nucleic acid construct according to one of the claims 12 to 16 and the cell or cells also comprise a nucleic acid sequence according to one of the claims 1 to 7 as promoter and a nucleic acid coding for a saccharose transport system, which is controlled by said promoter.

36. Plant according to claim 35, characterized in that in at least one cell and preferably in most of its cells, it comprises a nucleic acid construct according to one of the claims 12 to 16 and the cell or cells also comprise a nucleic acid sequence according to one of the claims 1 to 7 as promoter and a nucleic acid coding for saccharose synthase and/or cytoplasmically expressed invertase and whose expression is controlled by the promoter.

37. Plant according to one of the claims 31 to 36, characterized in that the further invertase differing from the cell's own invertase is selected from the group of invertases comprising invertases of *Saccharomyces cerevisiae* and invertases of *Zymomonas mobilis*.

38. Plant according to one of the claims 33 to 37, characterized in that the saccharose synthase is of a heterologous or homologous origin.

39. Plant according to one of the claims 34 to 38, characterized in that the cytoplasmically expressed invertase has a homologous or heterologous origin.

40. Plant according to claim 39, characterized in that the cytoplasmically expressed invertase has a heterologous origin and is preferably selected from the group of invertases comprising invertases of *Saccharomyces cerevisiae* and invertases of *Zymomonas mobilis*.

41. Seed obtained from a plant according to one of the claims 31 to 40.

42. Use of seed according to one of the preceding claims for the in vitro embryogenesis of haploid or diploid or double diploid plants.

43. Fruit, particularly seedless fruit, obtained from a plant according to one of the claims 22 to 24.

44. Fruit, obtained from a plant according to one of the claims 31 to 40.

45. Process for cloning promoters, which are functionally homologous to one of the promoters according to one of the preceding claims, characterized by the following steps:

- c) cloning of anther-specific invertase cDNA by RT-PCR on mRNA from anthers, particularly using the oligonucleotides OIN3 and OIN4,
- d) cloning the corresponding promoters.